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Analytical Method Development for Inhalation Formulation by using RP-HPLC

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ABSTRACT:

In the research analysis a rapid, accurate and reliable High Performance Liquid Chromatography (HPLC) method was developed and validated by selecting chromatographic parameters for estimation of SaIbutamoI, CiprofIoxacin, and MannitoI in pharmaceutical dosage forms. The HPLC method was developed using reverse phase technique and InertsiI column (C18 x 250 mm x 4.6 mm x 5 .0 u) with HPIC System like AgiIent 1260 Infinity. Detector like Photodiode array G7115A and the lambda max value at 277nm. This method has been validated by the use of different validation parameters such as accuracy, precision, linearity, lod and loq. Such findings showed that the system could find practical use in its tablet dosage forms as a quality assurance tool for evaluating the drug in pharmaceutical industries.

KEYWORDS: Method development, Validation, RP-HPLC, Pharmaceutical dosage forms.

INTRODUCTION:

Salbutamol Sulphate is a beta-2 adrenergic receptor agonist. It provides rapid and short-acting bronchodilation in reversible airway obstruction. It is used to treat bronchospasm caused by Asthma, COPD, and other lung diseases. In the respiratory tract, its effect is in the form of bronchial smooth muscle relaxation. Ciprofloxacin belongs to the class of drugs called fluoroquinolone antibiotics. It is used to treat several bacterial infections, e.g., respiratory tract infections and urinary tract infections, among others. Its mode of action is by inhibiting enzymes topisomerase-II and topoisomerase-IV required for bacterial DNA replication. Mannitol is a naturally occurring sugar that helps rehydrate mucus and airway clearances due to its osmotic properties.

Experimental work

Method development activity included HPLC trials for optimizing the chromatographic conditions for detection of Related substances (RS) in the combination product containing Salbutamol, Ciprofloxacin, and Mannitol. Two different methods were chosen as the difference in concentration or rather the low concentration of Salbutamol and even lower concentration of its impurities demanded a more sensitive detector than the RID. The developed method was suitable for both the test of Assay and Related substances of Salbutamol and Ciprofloxacin. Further, Mannitol assay method has already being developed in RID (in chapter 3), which was more productive as Related substances for mannitol are quantified in the same method.

HPLC Method Development and Validation for Assay and Related Substances of Salbutamol and Ciprofloxacin in the formulation.

HPLC Method Development and Validation for Related Substances of Mannitol in the formulation.

Chromatographic conditions:

Column : Inertsil C18 x 150 mm x 4.6 mm x 5 .0 u

Mobile Phase : Buffer : Acetonitrile (80: 20)

Buffer : 1.8 g Hexane sulphonic acid sodium salt + 2.5 g dihydrogen phosphate in 1000 ml water, adjust pH to 4.5 with dilute orthophosphoric acid

Diluent : Mobile Phase

Flowrate : 1.0 ml/min

Detector : UV detector, Wavelength 225 nm.

Injection Volume : 20 µl

Column temp. : 30 °C

Run time : 30 min

Elution mode : Isocratic

Precision:

Preparation of Assay standard solution

Assay – Standard stock solution A: About 9.5 mg of Salbutamol sulphate and about 116 mg of Ciprofloxacin Hydrochloride monohydrate were weighed in a 100 ml flask and dissolved in 70 ml diluent by sonication and made up to the mark.

Assay – Standard solution: 5.2 ml of stock solution was transferred to a 10 ml volumetric flask was made up to the mark to give a solution containing 40 µl/ml of Salbutamol and 520 µl/ml of Ciprofloxacin.

Preparation of Related substances standard solution

Salbutamol Stock solution: About 9.5 mg of Salbutamol sulphate was weighed in a 100 ml flask, dissolved in 70 ml diluent by sonication, and made up to the mark; 5.2 ml was transferred to a 10 ml flask and diluted up to the mark.

Ciprofloxacin Stock solution: About 116 mg of Ciprofloxacin Hydrochloride was weighed in a 100 ml flask, dissolved in 70 ml diluent by sonication, and made up to the mark, 5.2 ml of this solution was transferred to a 10 ml flask and diluted up to the mark.

Standard solution – Related substances

1 ml of Salbutamol stock solution and 0.5 ml of Ciprofloxacin stock solution were transferred to a 100 ml flask and diluted up to the mark to give a solution containing 0.4 µl/ml of Salbutamol and 2.6 µl/ml of Ciprofloxacin.

Preparation of Sample solution: Assay and RS

Due to the large difference in concentration of Salbutamol and Ciprofloxacin in the formulation the sample solution was made in two dilutions. The first one for Salbutamol and the second dilution for Ciprofloxacin.

Salbutamol sample solution: About 236 mg of sample blend was transferred in a 10 ml volumetric flask, dissolved in 7 ml diluent and diluted up to the mark to give a solution containing 40 µl/ml of Salbutamol.

Ciprofloxacin sample solution: 2 ml of the above solution was transferred in a 25 ml volumetric flask and diluted up to mark to give 520 µl/ml solution of Ciprofloxacin.

Table 1: Results for Precision Repeatability- Assay

	Salbutamol		**Ciprofloxacin**	
Sr. No.	**Precision**	**Intermediate Precision**	**Precision**	**Intermediate Precision**
1	94.99	97.09	97.06	98.44
2	94.97	97.25	96.60	98.47
3	94.68	95.81	96.48	98.70
4	94.80	96.64	96.48	98.89
5	94.99	97.69	96.86	98.66
6	94.90	96.74	96.42	98.69
Individual Mean	**94.9**	**96.87**	**96.65**	**98.69**
SD	0.13	0.64	0.26	0.21
% RSD	0.1	0.7	0.3	0.2
MEAN value difference	1.97		2.04	
Acceptance Criteria			RSD <= 2 %	

Linearity:

Linearity test for Assay was performed on five concentration level between 50 and 150 % of the test concentration. Six replicates injected for 50 % and 150 % level. For the test of Related substances, the linearity was demonstrated by injecting six concentration levels of Salbutamol, Salbutamol Imp D, Ciprofloxacin, and Ciprofloxacin Imp C in the range of LOQ to 150 %. Six replicates injected for LOD and 150 % level. The peak areas were plotted against respective concentrations. The linearity of the method was evaluated by using a calibration curve to calculate the coefficient of correlation and intercept values.

Linearity Solutions (Assay)

Linearity Stock Solution (Salbutamol and Ciprofloxacin – 77 and 1000 ppm)

Weigh and transfer 9.3 mg Salbutamol sulphate and 116.5 gm to Ciprofloxacin in 100 ml flask, dissolve and make up to volume 77.1 ppm and 1000 ppm resp.).

Salbutamol and Ciprofloxacin linearity solutions

The linearity was studied from 50% to 150% level of Salbutamol and Ciprofloxacin. Linearity solutions were prepared by addition of 2.6, 4.2, 5.2, 6.2, 7.8ml stock solution to 10 ml and diluted to volume, Corresponding to 20,32,40,48 and 60 ppm of Salbutamol and 260, 420, 520,620 and 780 ppm of Ciprofloxacin. Six injections of 50 % level and 150 % were made. Single injections of 80%, 100%, and 120% were made. A graph of concentration Vs Area was plotted and Correlation coefficient for the peaks was calculated.

Linearity Solutions (Related Substances)

Linearity Stock Solution (Salbutamol and Salbutamol impurity D - 100 ppm)

Weigh and transfer 12 mg Salbutamol sulphate in 100 ml flask, dissolve and make up to volume 100 ppm). Weigh and transfer about 1 mg of Salbutamol impurity D in 10 ml flask, dissolve and make up to volume (100 ppm). 2 ml of each solution in 20 ml flask and make up to volume (10 ppm).

Salbutamol and Salbutamol impurity D linearity solutions

The linearity was studied from 30 % (LOQ) to 150% level of Salbutamol and Salbutamol impurity D. Linearity solutions were prepared by addition of 0.3,0.5,0.8,1.0,1.2,1.5 ml of stock solution to 25 ml and diluting up to mark. 3.3 ml from LOQ was diluted to 10 ml for LOD concentration. Corresponding to 0.04, 0.12,0.2,0.32,0.40, 0.48, and 0.6 ppm (LOQ to 150%). Six injections of LOQ level and 150 % were made. Single injections of 50 %, 80%, 100%, and 120% were made. 3 injections of LOQ level were also made. A graph of c0ncentration Vs Area was plotted and Correlation coefficient for the peaks was calculated.

Table 2: Result for Linearity: Assay

Sr.No.	**Conc. Level (%)**	**Salbutamol**		**Ciprofloxacin**	
		ppm (X value)	**Area (Y Value)**	**ppm (X value)**	**Area (Y Value)**
1	50	20	370.3	260.2	4496.4
2	80	32.2	593.5	420.3	7258.9
3	100	39.9	744.1	520.3	9104.4
4	120	47.6	881.9	620.4	10798.7
5	150	59.9	1115.3	780.5	13735.4

Limit of Detection and Limit of Quantification

LOD and LOQ were determined by performing a series of injections of progressively diluted samples. The LOD and LOQ were determined at the lowest concentration, where the peak response ratio with the baseline noise would be >= 3:1 and 10:1 resp. LOD ppm was 0.04 for Salbutamol and Salbutamol impurity D and 0.17 for Ciprofloxacin and Ciprofloxacin impurity. LOQ ppm was 0.12 for Salbutamol and Salbutamol impurity D and 0.5 for Ciprofloxacin and Ciprofloxacin impurity.

Table 3: Results for LOD:

	Salbutamol		Salbutamol Imp D		Ciprofloxacin		Ciprofloxacin Imp C	
Sr. No	Area	S/N Ratio	Area	S/N Ratio	Area	S/N Ratio	Area	S/N Ratio
1	0.816	50.4	0.71	18.3	2.792	5.2	0.866	24.2
2	0.848	50.8	0.663	18.1	2.453	4.7	0.883	26.8
3	0.852	50.3	0.669	17.5	2.509	5.1	0.868	26.3
Mean	0.839	**50.5**	0.681	**18.0**	2.585	**5.0**	0.872	**25.77**
SD	0.020	0.265	0.026	0.416	0.18	0.26	0.009	1.38
% RSD	2.4	0.5	3.8	2.3	7.0	5.3	1.1	5.4

Acceptance Criteria: Limit of detection should have Signal to noise ratio above 3.

Accuracy: Assay

Stock solution preparation

Accuracy Stock Solution (Salbutamol and Ciprofloxacin 77 and 100 ppm)

Weigh and transfer 9.3 mg Salbutamol sulphate and 116.5 mg Ciprofloxacin Hydrochloride in 100 ml flask, dissolve and make up to volume.

Accuracy sample solution:

Accuracy sample solutions were prepared by addition of 2.6, 5.2, 7.8 ml of stock solution to 400 mg of placebo and diluting to10 ml. Corresponding to 20, 40, 60 ppm of Salbutamol and 260, 520,780 ppm of Ciprofloxacin. 3 injections per level were made.

Robustness:

Stability of analytical solution performed to study the stability of standard and sample solution stored at 8ºC. Prepared Standard solution and Sample solution as per the method, and injected these solutions into HPLC system at regular interval until the area of solution is within the acceptance criteria. Standard and Sample were prepared as per described in method precision.

Table 4: Stability of Analytical Solutions – Assay standard

Time (Hrs)	Salbutamol			Ciprofloxacin		
	Area Response	Average	% RSD	Area Response	Average	% RSD
Initial	742.024	-		9020.112	-	
5 hr.	743.924	742.974	0.2	9016.916	9018.514	0.0
12 hr.	745.246	743.635	0.3	9030.297	9025.205	0.1
18 hr.	743.423	742.724	0.1	9055.987	9038.050	0.3
26 hr.	743.055	742.540	0.1	9110.017	9065.065	0.7

Results and Evaluation

The stability of the standard solution and test solution is established up to minimum 26 hours.

b) Influence of variation of test parameters

This Robustness study is performed to study the reliability of test method for deliberate changes in chromatographic conditions. Robustness was studied for the following two parameters.

i. Flow rate up to ± 10 %

ii. Variability from column initial temperature ± 5ºC

Table 5: Result for Robustness - Assay

	Salbutamol	Abs. difference	Ciprofloxacin	Abs. difference
As such condition: Assay	94.99		97.06	
Change in flowrate: 0.8 ml	97.21	0.02	98.66	0.02
Change in flowrate: 1.2 ml	97.23	0.02	98.55	0.02
Change in temperature: 25 ° C	97.27	0.02	98.67	0.02
Change in temperature: 35 ° C	96.99	0.02	98.19	0.01

Validation Summary:

Table 6: Method Validation Summary : Salbutamol and Ciprofloxacin Assay and RS

Validation parameter	Units	Assay		Related Substances			
		Salbutamol	Ciprofloxacin	Salbutamol	Sal. Imp D	Ciprofloxacin	Cipro. Imp C
Specificity							
Specificity	RT	5.2	19.2	5.2	14.9	19.2	14.0
Response Factor		-	-	-	1.7	-	0.9
System suitability							
Retention time	mins	5.37	20.51	5.37	-	20.73	-
Tailing Factor	< 2	1.1	1.4	1.1	-	1.6	-
Theoretical plates	NLT 2000	10473	11578	10451	-	11195	-
Limit of detection	PPM	-	-	0.04	0.04	0.17	0.17
	S/N	-	-	50.5	18.0	5.0	25.8
Limit of quantification	PPM	-	-	0.12	0.12	0.5	0.5
	S/N	-	-	77.7	27.9	22.0	69.0
Linearity							
Linearity range	(ppm)	20 - 60	260 – 780	0.1 – 0.6	0.1 – 0.6	0.5 – 3.9	0.9 – 3.9
Correlation Coefficient	r2	0.999	0.999	0.999	0.999	0.999	0.999
Precision							
Precision	Mean	94.9	96.7	-	0.98	-	0.04
	% RSD	0.1	0.3	-	5.4	-	8.7
Int. Precision	Mean	96.9	98.7	-	1.19	-	0.03
	% RSD	0.7	0.2	-	9.3	-	1.9
Accuracy							
LOQ (n=3)	% Recovery	-	-	103.9	98	100.9	95.9
	% RSD	-	-	1.8	5.1	3.7	6.5
Ass. (80%) / RS (50 %) level	% Recovery	102.2	101.9	103.6	101.9	110.7	96.7
	% RSD	0.2	0.1	3.9	2.8	0.5	8.3

CONCLUSION:

In the present study, an attempt was made to develop a simple, accurate, precise, rapid and sensitive method was developed for the simultaneous estimation of the salbutamol sulphate, ciprofloxacin and mannitol in bulk and dosage form. Retention time of salbutamol was found to be 5.17 min and for ciprofloxacin is 4.16 min. %RSD of salbutamol is 0.7. % Recovery was obtained as 99.69%-99.96% and 100.12%-100.41% for salbutamol and ciprofloxacin, respectively. Retention times were decreased, and that run time was decreased, so the method developed was simple and economical, which is useful in pharmaceutical industries.

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